Effect of exercise and Lycium barbarum extract on serum Fe level and liver antioxidant enzyme activities rats fed with Fe-rich diet

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Lycium barbarum is known for its potent antioxidative and anti-inflammatory effects. To study whether L.. barbarum extract can modulate serum Fe level and oxidative injury, thirty-two male Sprague-Dawley rats were divided into six groups. Normal control (Group I): fed a balanced diet and kept sedentary (untrained) until the end of the experiment; Model Control (Group II): fed a Fe-rich diet and kept sedentary (untrained) until the end of the experiment; Training group (Group III): fed a Fe-rich diet and submitted to swimming training (1 h daily) for 2 weeks; LBCE group (Group VI): fed a Fe-rich diet, received lycium barnarum carotenoid extract (200 mg/kg b.w. day) and submitted to swimming training (1 h daily) for 2 weeks. Results showed that LBCE could enhance Fe absorption and decrease liver oxidative damage in rats. In conclusion, the present study demonstrates for the first time that there a synergistic effects (LBCE and Fe) against liver oxidative damage in rats.

Key words: Exercise, Fe, liver, Lycium barbarum, rat.

INTRODUCTION

Lycium fruits appear in Chinese lore as far back as 2800 B.C. in association with the legendary First Emperor, Shen Nung, who was an herbalist and the mythical father of agriculture. The ancient herbalist classics recorded that L. barbarum nourishes the liver and kidney and brightens the eye. In his “Compendium of Medica”, Li Shi-zen named L. barbarum as a top-grade medicinal material that can nourish the liver and kidney, supplement energy and improve eyesight. “Shennong’s Classic of Materia Medica (Shennong Bencaojing)” also mentioned that “long term use of goji can contribute to agility and longevity.” Ni Zhu-Mo, the renowned Chinese herbalist, also said in his “Ben Cao Hui Yan (Convergent Speech on the Materia Medica)” that “Goji can supplement energy, blood, adjust Yin and Yang, reduce internal heat and resist wind and humidity, and enjoys ten magic functions” (Wang, 2006). Ethnobotanists have found that Lycium is still used by healers in Israel (Dafni and Yaniv, 1994). Exercise is postulated to generate free radicals by other means, including; (1) increases in epinephrine and other catecholamines that can produce oxygen radicals when they are metabolically inactivated, (2) production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl), and (3) inflammatory responses to secondary muscle damage incurred with overexertion (Aruoma, 1994; Ji, 1995; Tiidus and Houston, 1995; Clarkson, 1995; Maxwell, 1995; Sen, 1995; Dekkers et al., 1996; Alessio, 1993; Packer, 1997; Ji et al., 1998; Ashton et al., 1998; Kanter, 1998). Regular exercise is known to increase the activity of antioxidant enzymes (Ji and Hollander, 2000), and hence increase protection against the activity of reactive oxygen and nitrogen species (RONS). Similarly to antioxidant administration regular exercise could also promote and/or retard the development of certain cancers, depending on the timing and perhaps on the intensity of the exercise (Thompson et al., 1995). Iron excess is a potent inducer of
peroxidation, which can overwhelm antioxidant defences and lead to tissue lesions; as such it contributes to the development of colon tumorigenesis (Nelson et al., 1989). However, there is no yet evidence that iron supplementation may improve performances in athletes, except in the case of iron deficiency (Nielsen and Nachtigall, 1998). High doses of iron are seldom used by athletes to increase haemoglobin synthesis, O2 transport and, ultimately, to improve performance (Major et al., 1997). Effective and promising molecules have been elaborated to treat severe anaemia, but they are sometimes used for doping purpose. An iron supplementation is usually added to these drugs to enhance erythropoietin-induced erythropoiesis (Major et al., 1997) and to prevent iron deficiency related to drug-induced increased erythropoiesis (Karltwasser, 1999). The present study was devoted to the assessment of the Fe-induced increased erythropoiesis (Karltwasser, 1999). The study was designed to evaluate the potential of L. barbarum carotenoid extract to enhance erythropoiesis and to prevent iron deficiency related to drug-induced iron deficiency (Nielsen and Nachtigall, 1998). However, there is no yet evidence that iron supplementation may improve performances in athletes, except in the case of iron deficiency (Nielsen and Nachtigall, 1998). High doses of iron are seldom used by athletes to increase haemoglobin synthesis, O2 transport and, ultimately, to improve performance (Major et al., 1997). Effective and promising molecules have been elaborated to treat severe anaemia, but they are sometimes used for doping purpose. An iron supplementation is usually added to these drugs to enhance erythropoietin-induced erythropoiesis (Major et al., 1997) and to prevent iron deficiency related to drug-induced increased erythropoiesis (Karltwasser, 1999). The present study was devoted to the assessment of the Fe level and oxidative stress induced by Fe-rich diet and protective effect of exercise and L. barbarum extract and carotenoid.

MATERIALS AND METHODS

Lycium barbarum fruits were purchased from a local herb shop in Lanzhou, China in March, 2011. Extract of L. barbarum was prepared in our laboratory, using traditional boiling water extraction method. Carotenoid was extracted from L. barbarum in our laboratory.

Animals

Thirty-two male Sprague-Dawley rats, mean (± SEM) weight 190 ± 5 g, were used for experiments. Rats were maintained at 23°C under pathogen-free conditions on a 12 h dark–light cycle and received food and water ad libitum. The present study was approved by the Animal Research Committee of our University.

Medicine treatment and exercise training

One week after weaning (28 days old), the animals were divided into 4 groups:

1. Normal control (Group I): fed a balanced diet and kept sedentary (untrained) until the end of the experiment;
2. Model Control (Group II): fed a Fe-rich diet and kept sedentary (untrained) until the end of the experiment;
3. Training group (Group III): fed a Fe-rich diet and submitted to swimming training (1 h daily) for 2 weeks;
4. LBCE group (Group VI): fed a Fe-rich diet, received lycium barnarum carotenoid extract (200 mg/kg b.w. day) and submitted to swimming training (1 h daily) for 2 weeks.

At the end of the experiment, all animals were sacrificed between 09:00 and 11:00 AM. Food was removed from the animals' cage at least 3 h before sacrifice. After complete anaesthesia (pentobarbital sodium, 50 mg/kg ip), the abdominal cavity was rapidly opened along the median line of the abdomen. Blood was rapidly (~<45 s) drawn from the abdominal vena cava (~4 ml) into syringes pretreated with EDTA (15%). Thereafter, blood was centrifuged (3,000 rpm for 10 min, 4°C). The liver median lobe was freeze-clamped and used for biochemical analysis.

Biochemical analysis

Nonheme iron content (NHI), Hb, and hematocrit (Hct) were measured according to the literature (Che et al., 2010). Plasma Fe, TIBC and Ts were measured using commercially available kits. Malondialdehyde (MDA), which is a measure of lipid peroxidation, was spectrophotometrically measured by using the thiobarbituric acid assay (Okawa et al., 1979; Padurariu et al., 2010). 200 µl of supernatant was added and briefly mixed with 1 ml of 50% trichloroacetic acid in 0.1 M HCl and 1 ml of 26 mM thiobarbituric acid. After vortex mixing, samples were maintained at 95°C for 20 min. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatants were read at 532 nm. A calibration curve was constructed using MDA as standard and the results were expressed as nmol/ml protein.

Reduced glutathione levels (GSH) were determined by Ellman method (1959) modified by Jollow et al. (1974) based on the development of a yellow colour when DTNB (5,5-dithiobis-2-nitrobenzoic acid) was added to compounds containing sulfhydryl groups. The absorbance was measured at 412 nm after 10 min. Total GSH content was expressed as µg/g tissue. Superoxide dismutase (SOD) activity was estimated according to the method of Beauchamp and Fridovich (1971). The developed blue colour in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as U/mg protein.

CAT activity was assayed by the method of Aebl (1984). In brief, to a quartz cuvette, 0.65 ml of the phosphate buffer (50 mmol/l; pH 7.0) and 50 µl sample were added, and the reaction was started by addition of 0.3 ml of 30 mM hydrogen peroxide (H2O2). The decomposition of H2O2 was monitored at 240 nm at 25°C. CAT activity was calculated as nM H2O2 consumed/min/mg of tissue protein.

The GPx activity assay was based on the method of Paglia and Valentine (1967). tert-Butylhydroperoxide was used as substrate. The assay measures the enzymatic reduction of H2O2 by GPx through consumption of reduced glutathione (GSH) that is restored from oxidized glutathione GSSG in a coupled enzymatic reaction by GR. GR reduces GSSG to GSH using NADPH as a reducing agent. The decrease in absorbance at 340 nm due to NADPH consumption was measured in a Molecular Devices M2 plate reader (Molecular Devices, Menlo. Park, CA). GPx activity was computed using the molar extinction coefficient of 6.22 mM−1 cm−1. One unit of GPx was defined as the amount of enzyme that catalyzed the oxidation of 1.0 µmol of NADPH to NADP+ per minute at 25°C.

Statistical analysis

Statistical analyses of the results were performed by using the two-way analysis of variance (ANOVA) and Bonferroni's post hoc test (GraphPad Prism 4, Graph Pad Software, Inc., San Diego, CA, USA). Results were considered statistically significant for P < 0.05.

RESULT AND DISCUSSION

Iron is critical to human life. It plays the central role in the hemoglobin molecule of our red blood cells RBC where it functions in transporting oxygen from the lungs to the body's tissues and carbon dioxide from the tissues to the lungs. Iron also functions in several key enzymes in energy production and metabolism including DNA synthesis. The present work demonstrated the lack of biochemical indexes changes in the blood and liver.
of rats when fed an Fe-enriched diet. Serum hemoglobin is a test that measures the level of free hemoglobin in the liquid part of the blood (the serum). Free hemoglobin is the hemoglobin outside of the red blood cells (D'Agnillo and Chang, 1998). HCT is one of the numbers you will see on your complete blood count (CBC) report. If your HCT numbers are not in a normal range, your treatment may be withheld or delayed until those numbers are better. Total iron binding capacity (TIBC) is a blood test that shows if there is too much or too little iron in the blood. Iron is carried in the blood attached to the protein transferrin. This test helps measure the ability of a protein called transferrin to carry iron in the blood (Vitoratos et al., 1999). Transferrin Saturation (TS) is the percent of transferrin that has iron bound to it. The normal range is 20-50%. It is the ratio of serum iron and total iron-binding capacity, multiplied by 100.

Transferrin saturation test (TS), a type of iron study (blood test) that measures the percentage of transferrin and other mobile, iron-binding proteins saturated with iron (Franzini et al., 2000). In the present study, Table 1 shows that the serum Hb, Hct, plasma Fe, TIBC and Ts levels were significantly higher in the Group II than in the Group I (P < 0.01). This result indicates that our model was successful. The training markedly (P < 0.05, P < 0.01) reduced serum Hb, Hct, plasma Fe, TIBC and Ts levels in the Group III compared to the Group II. However, the serum Hb, Hct, plasma Fe, TIBC and Ts levels were significantly enhanced in the Group IV rats compared to Group III (P < 0.05, P < 0.01).

Table 2 shows that the liver NHI level was significantly higher in the Group II than in the Group I (P < 0.01). The training markedly (P < 0.01) reduced liver NHI level in the Group III compared to the Group II. However, the liver NHI level was significantly enhanced in the Group IV rats compared to Group III (P < 0.01). These data indicated that LBCE supplement could dietary Fe absorption in these animals. These results confirm the aforementioned literature data. As shown in Table 3, there was no significantly difference in liver index between groups (P > 0.05).

A number of methods have been established as markers of oxidative stress in animal cells, caused either by excessive production of ROS or reduced antioxidant defense (Armstrong, 2002). Lipid peroxidation represents oxidative tissue damage caused by hydrogen peroxide, superoxide anion and hydroxyl radicals, resulting in structural alteration of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products. Malondialdehyde is a major end product of free radical reaction on membrane fatty acids. Although, the cell is
endowed with several antioxidant systems to limit the extent of lipid peroxidation, under certain conditions protective mechanism can be overwhelmed, leading to elevated tissue levels of peroxidation products (Candan and Tuzmen, 2008).

As shown in Table 4, MDA level induced by Fe-rich diet. Rats in Group II developed high degree of MDA level compared to Group I. Training further increased liver MDA level (Group III). Administration of LBCE diminished lipid accumulation very noticeably (Group IV).

Superoxide dismutases (SOD) are metalloenzymes that catalyze the dismutation of superoxide anion (•O₂⁻) into O₂ and hydrogen peroxide (H₂O₂) (Fridovich, 1986). The Cu/Zn-SOD is localized in the cytosol and nucleus, while Mn-SOD is located within the mitochondrial matrix. Subsequently, H₂O₂ is reduced to H₂O by glutathione peroxidase (GSH-Px) in the cytosol, or by catalase (CAT) in the peroxisomes. SOD, CAT and GSH-Px, together with GSH-S-transferase and GSH reductase, are easily induced by oxidative stress, and the activity levels of these enzymes have been used to quantify oxidative stress in cells (van et al., 2003). Glutathione and GSH disulfide (GSSG) are biologically important intracellular thiols, and alterations in the ratio between total GSH (tGSH) and GSSG (oxidative stress index) are also often used to assess exposure of cells to oxidative stress. Tissue markers of lipid peroxidative stress include reduced vitamin E (α-tocopherol) content and increased thiobarbituric-reactive substances (TBARS) (Berntssen et al., 2000; Armstrong, 2002), and water hyper oxygenation may be lower when dietary iron is present in a polyphenol-containing meal. However, our present work suggested that LBCE did not decrease iron absorption. In addition, LBCE still reduced oxidative injury and enhanced antioxidant enzymes activities in animals.

**REFERENCES**


